

# BEST AVAILABLE COPY

Application No. 10/053,530

Docket No. 30906/41458UTL2

## Amendment to the claims

This listing of claims will replace all prior versions, and listings, of claims in the application:

1-22. (canceled)

23. (previously presented) A single chain protein, comprising:

(a) a binding domain polypeptide capable of binding to a target, said binding domain polypeptide being joined to

(b) a hinge peptide, said hinge peptide being joined to

(c) an immunoglobulin heavy chain CH2 constant region polypeptide, said CH2 constant region polypeptide being joined to

(d) an immunoglobulin heavy chain CH3 constant region polypeptide,

wherein said hinge peptide is an IgG or IgA hinge peptide that has been made to contain one or two cysteine residues, provided that when the hinge peptide contains two cysteines the first cysteine of the hinge that is responsible for forming a disulfide bond with a light chain constant region in a naturally-occurring IgG or IgA antibody is not deleted or substituted with an amino acid, and

wherein said single chain protein (1) is capable of binding to said target, and (2) is capable of promoting antibody dependent cell-mediated cytotoxicity or complement fixation or both.

24. (previously presented) A single chain protein, comprising:

(a) a binding domain polypeptide capable of binding to a cell surface receptor, said binding domain polypeptide being joined to

(b) a hinge peptide, said hinge peptide being joined to

(c) an immunoglobulin heavy chain CH2 constant region polypeptide, said CH2 constant region polypeptide being joined to

(d) an immunoglobulin heavy chain CH3 constant region polypeptide,

wherein said hinge peptide is an IgG or IgA hinge peptide that has been made to contain one or two cysteine residues, provided that when the hinge peptide contains two cysteines the first cysteine of the hinge that is responsible for forming a disulfide bond with a light chain constant region in a naturally-occurring IgG or IgA antibody is not deleted or substituted with an amino acid, and

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wherein said single chain protein is capable of antibody dependent cell-mediated cytotoxicity or complement fixation or both.

25. (previously presented) A single chain protein, comprising:

(a) a binding domain polypeptide capable of binding to a target cell, said binding domain polypeptide being joined to

(b) a hinge peptide, said hinge peptide being joined to

(c) an immunoglobulin heavy chain CH2 constant region polypeptide, said CH2 constant region polypeptide being joined to

(d) an immunoglobulin heavy chain CH3 constant region polypeptide,

wherein said single chain protein is capable of binding to said target and decreasing the number of target cells, and

wherein said hinge peptide is an IgG or IgA hinge peptide that has been made to contain one or two cysteine residues, provided that when the hinge peptide contains two cysteines the first cysteine of the hinge that is responsible for forming a disulfide bond with a light chain constant region in a naturally-occurring IgG or IgA antibody is not deleted or substituted with an amino acid.

26. (previously presented) The single chain protein of any one of claims 23, 24, or 25 wherein said binding domain polypeptide is a single chain Fv polypeptide.

27. (previously presented) The single chain protein of claim 26 wherein said single chain protein is capable of binding to a B cell target.

28. (previously presented) The single chain protein of claim 27 wherein said B cell target is CD20.

29. (previously presented) The single chain protein of claim 27 wherein said B cell target is CD37.

30. (previously presented) The single chain protein of claim 27 wherein said B cell target is selected from the group consisting of CD19, CD22, CD30 ligand, CD54, CD106, and interleukin-12.

31. (previously presented) The single chain protein of claim 27 wherein said single chain protein is capable of depleting a population of target cells.

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32. (previously presented) The single chain protein of claim 25 wherein said single chain protein is capable of decreasing the number of target cells in vivo.

33. (previously presented) The single chain protein of claim 25 wherein said single chain protein is capable of decreasing the number of target cells in vitro.

34. (previously presented) The single chain protein of claim 26 wherein the heavy and light chain variable regions of the single chain Fv are joined by a polypeptide linker of at least about 6 amino acids.

35. (previously presented) The single chain protein of claim 25 wherein said binding domain is a single chain Fv polypeptide capable of binding to a target selected from the group consisting of CD2, CD5, CD10, CD27, CD28, CD40, CTLA-4, 4-1BB, 4-IBB ligand, interferon- $\gamma$ , interleukin-4, interleukin-17, and interleukin-17 receptor.

36. (previously presented) The single chain protein of claim 25 wherein said binding domain is a single chain Fv polypeptide capable of binding to a target selected from the group consisting of CD59, CD48, CD72, CD70, CD86/B7.2, CD40 ligand, IL-17, CD43 and VLA-4 ( $\alpha_4\beta_7$ ).

37. (previously presented) The single chain protein of claim 25 wherein said binding domain is a single chain Fv polypeptide capable of binding to a target selected from the group consisting of CD83 and DEC-205.

38. (previously presented) The single chain protein of claim 25 wherein said binding domain is a single chain Fv polypeptide capable of binding to a target selected from the group consisting of HER1, HER2, HER3, HER4, epidermal growth factor receptor, vascular endothelial cell growth factor, vascular endothelial cell growth factor receptor, insulin-like growth factor-I, insulin-like growth factor-II, transferrin receptor, estrogen receptor, progesterone receptor, follicle stimulating hormone receptor, retinoic acid receptor, MUC-1, NY-ESO-1, NA 17-A, Melan-A/MART-1, tyrosinase, Gp-100, MAGE, BAGE, GAGE, CTA class receptors, the HOM-MEL-40 antigen encoded by the SSX2 gene, carcinoembryonic antigen, and PyLT.

39. (previously presented) The single chain protein of any of claims 23, 24 or 25 wherein said binding domain polypeptide is a single chain Fv capable of binding CD20, wherein said hinge peptide contains one or two cysteines that have been deleted or substituted

with non-cysteine amino acid residues, and wherein said immunoglobulin heavy chain CH2 and CH3 constant region polypeptides are IgG1 CH2 and CH3 constant region polypeptides.

40. (previously presented) The single chain protein of claim 39, wherein said single chain protein includes a 2H7 single chain Fv binding domain polypeptide.

41. (previously presented) The single chain protein of claim 39, wherein said single chain protein includes a 2H7 single chain Fv binding domain polypeptide, and wherein said hinge peptide contains one or more serine in place of one or more cysteine residues.

42. (Currently amended) The single chain protein of claim 39, wherein said heavy chain constant region comprises a CH2 domain in which a leucine has been replaced with serine at position 234.

43. (previously presented) The single chain protein of claim 42, wherein the binding domain polypeptide is a 2H7 single chain Fv.

44. (previously presented) The protein of claim 5 wherein said binding domain polypeptide is a 2H7 single chain Fv, and wherein said hinge peptide comprises at least a portion of an IgA hinge.

45-46. (canceled)

47. (previously presented) The single chain protein of claim 46 wherein said hinge peptide comprises a wild type IgA hinge.

48. (previously presented) The single chain protein of any of claims 26 wherein said binding domain is an single chain Fv capable of binding a L6 carcinoma antigen, said hinge peptide contains one or two cysteines that have been deleted or substituted with non-cysteine amino acid residues, and said immunoglobulin heavy chain CH2 and CH3 constant region polypeptides are IgG1 CH2 and CH3 constant region polypeptides.

49-101. (canceled)

102. (previously presented) The single chain protein of claim 26 wherein said single chain Fv polypeptide is a 2H7 scFv, wherein said hinge peptide comprises at least a portion of an IgA hinge.

103. (previously presented) The single chain protein of claim 102 wherein said hinge peptide comprises a wild type IgA hinge.

104. (previously presented) The single chain protein of claim 26 wherein said target is an L6 carcinoma antigen, said binding domain is capable of binding L6, said hinge peptide comprises at least a portion of an IgA hinge, and said immunoglobulin heavy chain CH2 and CH3 constant region polypeptides are from IgG1.

105. (previously presented) The single chain protein of claim 104 wherein said hinge peptide comprises a wild type IgA hinge.

106. (previously presented) The single chain protein of claim 26 wherein said target is an L6 carcinoma antigen, said binding domain is capable of binding L6, one or more cysteine residues in said hinge peptide have been replaced with one or more serine residues, and said immunoglobulin heavy chain CH2 and CH3 constant region polypeptides are from IgG1

107-141. (canceled)

142. (previously presented) The single chain protein of claim 39 wherein one or both of said IgG1 CH2 and CH3 constant region polypeptides are human IgG1 CH2 and CH3 constant region polypeptides.